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A comparative study of three tissue-cultured *Dendrobium* species and their wild correspondences by headspace gas chromatography–mass spectrometry combined with chemometric methods

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ABSTRACT

Plant tissue culture technique is widely used in the conservation and utilization of rare and endangered medicinal plants and it is crucial for tissue culture stocks to obtain the ability to produce similar bioactive components as their wild correspondences. In this paper, a headspace gas chromatography–mass spectrometry method combined with chemometric methods was applied to analyze and evaluate the volatile compounds in tissue-cultured and wild *Dendrobium huoshanense* Cheng and Tang, *Dendrobium officinale* Kimura et Migo and *Dendrobium moniliforme* (Linn.) Sw. In total, 63 volatile compounds were separated, with 53 being identified from the three *Dendrobium* spp. samples. Different provenances of *Dendrobiums* had characteristic chemicals and showed remarkable quantity discrepancy of common compositions. The similarity evaluation disclosed that the accumulation of volatile compounds in *Dendrobium* samples might be affected by their provenance. Principal component analysis showed that the first three components explained 85.9% of data variance, demonstrating a good discrimination between samples. Gas chromatography–mass spectrometry techniques, combined with chemometrics, might be an effective strategy for identifying the species and their provenance, especially in the assessment of tissue-cultured *Dendrobium* quality for use in raw herbal medicines.

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1. Introduction

Dendrobium, a precious traditional Chinese medicine, has been used in the preparation of herbal medicines in China for more than 2000 years. Sections of the stems of *Dendrobiums* have long been used to cure throat inflammation, nourish the stomach, promote secretion of saliva or as a tonic to promote the production of body fluid and improve the quality of life [1]. Seventy-four species of *Dendrobium* and two varieties are found in China [2]. The slow growth rate and excessive harvesting had left some of them critically endangered, especially *Dendrobium huoshanense* [3].

Plant tissue culture technique is widely used in the conservation and utilization of rare and endangered medicinal plants due to its remarkable ability of quickly increasing their biomass [4,5]. In the traditional product region, tissue-cultured dendrobiums have already become the major resource of pharmaceutical *Dendrobiums*. It is vital for tissue culture stocks to obtain the ability to produce similar bioactive components as their wild correspondences besides keeping genetic information and morphologies homoplastic between different provenances. Therefore, establishing a fast, quality identification method to evaluate the chemical similarity of the wild and tissue-cultured *Dendrobium* is a critical step for assurance of quality and safety in the traditional Chinese medicine industry.

The volatile components of herbal medicines contain a significant number of compounds and are used as markers for authenticity. The variations of volatile components in plants might be caused by differences in species, habitats, variety, cultivation patterns, or the extraction and analysis methods applied for composition determination [6,7]. Accordingly, it might be practical to establish a gas chromatography–mass spectrometry (GC–MS) fingerprint method based on the analysis of the volatile components to evaluate the similarity between tissue-cultured medicinal plants and their wild correspondences.

In this paper, we aimed to apply the headspace GC–MS technique coupled with a series of chemometric methods to fingerprint the volatile compounds from the stems of tissue-cultured and wild *D. huoshanense*, *Dendrobium officinale*, and *Dendrobium moniliforme*. To our knowledge, no documents have ever mentioned the discrimination and similarity evaluation of the tissue-cultured and wild *Dendrobium* plants by GC–MS method. Therefore, our study might be beneficial for developing a rapid, feasible and economical tool based on GC–MS for the identification and quality evaluation between different provenances of *Dendrobium* species, and provide new insights into the utilization and conservation of rare and endangered medicinal plants by tissue culture techniques.

2. Methods

2.1. In vitro *callus growth protocol, plant materials, and chemicals*

The *in vitro* plantlets of the three tissue-cultured dendrobiums were regenerated via protocorm-like bodies in the laboratories of West Anhui Biotechnology Research Center of Natural Medicine and Traditional Chinese Medicine and were then transplanted in the cultivated base in Huoshan count, Anhui Province, China. The current season's vegetative stems of tissue-cultured and wild *D. huoshanense*, *D. officinale*, and *D. moniliforme* were collected in October 2013, from Huoshan County, Anhui Province, China. All the plant materials were identified by Professor Nai-Fu Chen, Anhui Biotechnology Research Center of Plant Cell Engineering, Anhui Province, China. The voucher specimens were deposited at the Herbarium, College of Biotechnology and Pharmaceutical Engineering, West Anhui University, Anhui Province, China (Table 1).

The authentic chemicals and alkane standard solutions of C8-C20 (mixture no. 115321-01-4PAK) were purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China).

The fresh collected *Dendrobium* samples were washed thoroughly in tap water and then freeze-dried by a Micro-Modulyo lyophilizer (Thermo Fisher Scientific, West Palm Beach, FL, USA), powdered in a blender and then every 2.0 g of dried sample was performed for headspace/GC–MS analysis.

2.2. *Equipment and conditions*

GC–MS analysis was performed with Trace 1300 gas chromatograph coupled to ISQ mass spectrometer (Thermo Fisher Scientific, West Palm Beach, FL, USA) series equipment including a TriPLUS RSH autosampler. The volatile compounds were separated on a TG-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Total program time was 39 minutes and the column oven temperature program was: 50°C (maintained for 1 minute) to 60°C at 1°C/min, to 200°C at 5°C/min. The carrier gas was Helium, 1.0 mL/min, split ratio 5:1, injector temperature 250°C. The samples were heated in the agitator oven for 10 minutes with constant incubation mode at 140°C. The injection volume was 2.5μL. The MS transfer line and ion source were at 280°C and 250°C, respectively. The MS mode was electron impact. The mass range scanned was 40–350 atomic mass units.

2.3. *Identification of the separated compounds*

The identification of the separated compounds was carried out by three different methods: (1) retention indices [8] of the compounds to be identified compared the retention index values detected by the same type of capillary column in the National Institute of Technology and Standards mass spectra libraries; (2) retention times of authentic standards in the same equipment and conditions; and (3) mass spectra, with indexes of relative match above 800 (US National Institute of Technology and Standards mass spectra libraries and also authentic chemicals). Compounds were marked as tentatively identified when identification was only based on mass spectral data.

All peaks found in at least two of the three total ion chromatograms (TIC) were taken into account when calculating the total area of peaks (100%) and the relative areas of the volatile compounds.

Table 1 – List of *Dendrobium* samples.

Samples	Abbreviation	Source	Voucher No.
Wild *Dendrobium huoshanense*	W-DHS	Huoshan, Anhui, China	201310HS0101Y
Tissue-cultured *D. huoshanense*	TC-DHS	Huoshan, Anhui, China	201310HS0101T
Wild *Dendrobium officinale*	W-DO	Huoshan, Anhui, China	201310HS0201Y
Tissue-cultured *D. officinale*	TC-DO	Huoshan, Anhui, China	201310HS0201T
Wild *Dendrobium moniliforme*	W-DM	Huoshan, Anhui, China	201310HS0301Y
Tissue-cultured *D. moniliforme*	TC-DM	Huoshan, Anhui, China	201310HS0301T

2.4. Quantitative analysis of the volatile compounds

According to the resolved chromatogram and mass spectra, the quantitative analysis of each component can be directly calculated by the overall volume integration [6,9–12]. They are proportional to the content of the peak as integration based on TIC when calculating the total area of peaks (100%) and the relative areas of the volatile compounds. The peak area in TIC was chosen as the analytical signal for the relative content. The precision and repeatability of the determination were established using six individual weighed powder samples. Relative standard deviations of peak areas of selected components were calculated.

2.5. Statistical analysis

The similarities of the fingerprint in the samples were evaluated by the correlation coefficient (r_{cor}) calculated by included cosine angle using SPSS software Version 16.0 (CAMO Software AS, Woodbridge, NJ, USA). For the characterization of the investigated *Dendrobium* samples, the obtained GC–MS profiles were subjected to principal component analysis (PCA) with MetaboAnalyst 3.0 [13,14].

3. Results and discussion

3.1. Fingerprinting of volatile components by GC–MS analysis

After resolution, according to the GC–MS analysis of the six *Dendrobium* samples, a total of 63 compounds were separated and 52 were identified.

The characteristic GC–MS chromatograms of tissue-cultured *D. huoshanense* (TC-DHS) and wild *D. huoshanense* (W-DHS), tissue-cultured *D. officinale* (TC-DO) and wild *D. officinale* (W-DO), tissue-cultured *D. moniliforme* (TC-DM) and wild *D. moniliforme* (W-DM) are presented in Figure 1, showing the specific volatile compound profiles from different *Dendrobium* samples, isolated using the headspace technique and analyzed by GC–MS. The main compounds were normal chain aliphatic alcohols and aldehydes, esters, aromatic compounds and terpene, the most abundant components were 1-decanol (in W-DHS, 41.4 ± 3.87%), furfural (in W-DO, 20.84 ± 1.75%), and 1-dodecene (in TC-DHS, 18.56 ± 2.35%; Table 2). The relative proportion of the three main compounds (expressed as a percentage of total peak area) accounted for over 30% of all volatile compounds found in the *Dendrobium* samples.

3.2. Semiquantitative GC–MS analysis

Semiquantitative GC–MS analysis, a common quantity method when standard chemicals are difficult to obtain [6,8,15], was applied to evaluate the contents of the separated compounds in the *Dendrobium* samples by calculating the overall volume integration in TIC. The peak area in TIC was chosen as the analytical signal for the relative content because it is proportional to the content of the peak as integration based on TIC when calculating the total area of peaks (100%) and the relative areas of the volatile compounds. The separated components are listed in Table 2 and expressed as percentages of total peak area (%). As can be observed, each *Dendrobium* had its own major compounds. In TC-DHS, the contents of 1-dodecene (42), octanoic acid (43), terpinolene (34), and benzaldehyde (26) were 16.33–20.79%, 12.97–15.18%, 9.65–13.91%, and 6.72–9.38%, respectively; W-DHS showed a distinctive characteristic of high content of 1-decanol (51), at over 45%, while only 0.69 ± 0.07% of 51 was detected in TC-DHS and no compound 51 was detected in *D. officinale* and *D. moniliforme* in our experimental conditions. The most abundant volatile compounds in TC-DO were propanamide, N,N-dimethyl- (4) (11.45–13.83%), Furfural (15) (11.14–12.36%), hexanal (10) (9.77–11.01%) and eucalyptol (28) (8.35–12.27%). W-DO had almost the same major compounds as those in its tissue-cultured correspondence. In TC-DM, the major compounds were benzeneacetaldehyde (34), hexanal (10), benzaldehyde (26), and cis-9-tetradecen-1-ol (28), while they were dodecanal (46), terpinolene (34), octanoic acid (43), benzaldehyde (26), and methyl isobutyl ketone (2) in W-DM.

Comparing volatile compounds profiles from the six investigated *Dendrobium* stocks, each sample had its characteristic volatile compounds. For example, the characteristic chemicals of TC-DHS were 2,3,5-trimethyl-phenol (50), n-tridecanol (61), and pentadecanoic acid (63) in contrast to W-DHS and the other two *Dendrobium* species. Similarly, the characteristic chemicals of TC-DO were 3-methyl-1-pentanol (16), 2-hexen-1-ol, (E)- (20), and 4,4-dimethyl-2-cyclohexen-1-one (25); TC-DM's characteristic chemicals were cyclohexene (1) and isolongifolene epoxide (49). The contents of some common volatile compounds varied remarkably between different provenances of the three dendrobiums. For example, TC-DHS had over 21% of 1-dodecene (42), about 10–40 times the contents in the other five *Dendrobium* samples. About 22% of furfural (15) was detected in W-DO, obviously higher than that in other samples. The content of terpinolene (34, 28.18 ± 1.2%) was more than twice that in W-DM. From the GC–MS spectra profiles, the characteristic chemicals and the contents of common volatile compounds into consideration, the six *Dendrobium* samples could be discriminated approximately.

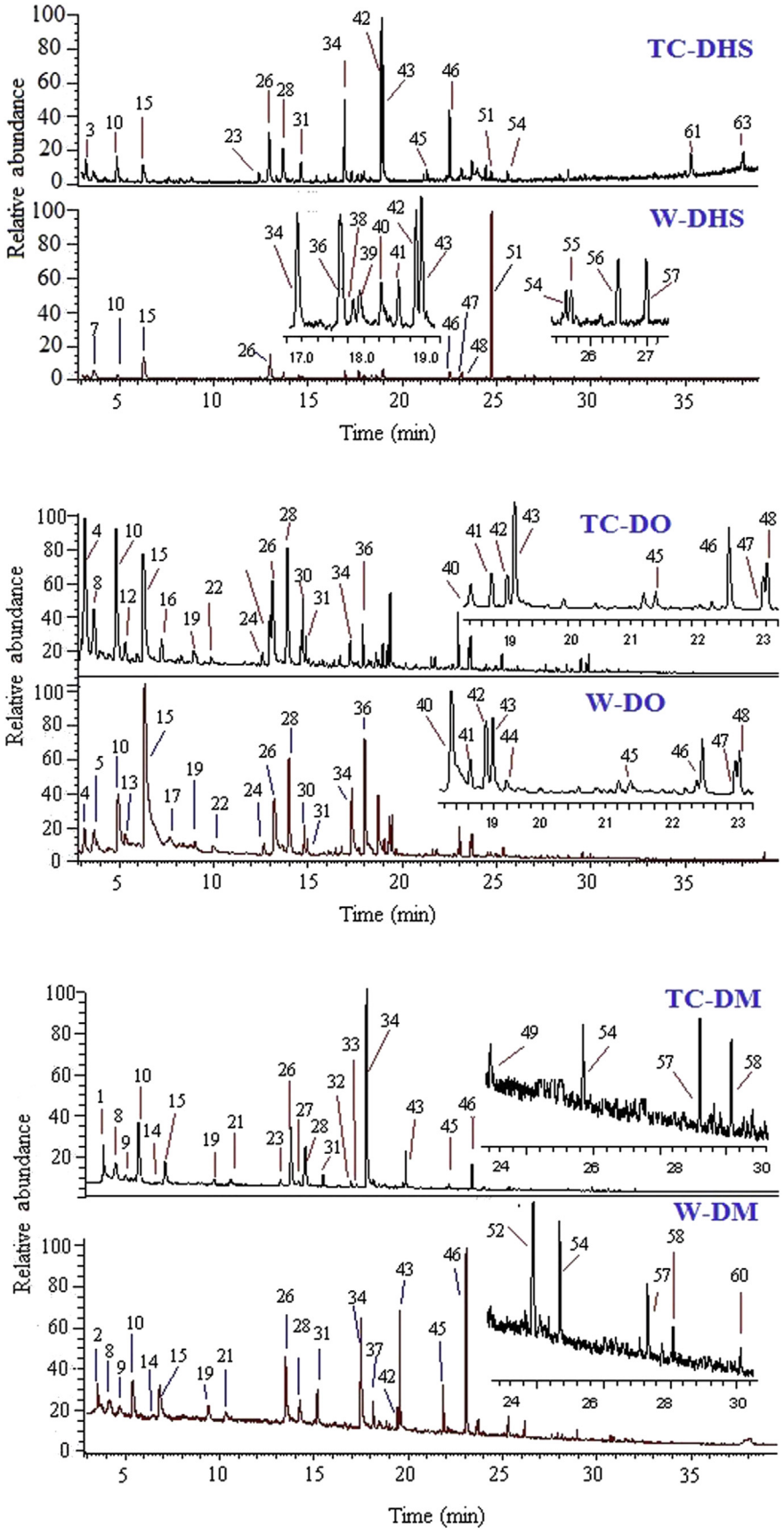

Figure 1 – Total ion chromatograms of headspace GC–MS analysis of volatile compounds from different provenances of tissue-cultured and wild *Dendrobium* varieties. The numbering refers to Table 2. TC-DHS = tissue-cultured *Dendrobium huoshanense*; TC-DM = tissue-cultured *Dendrobium moniliforme*; TC-DO = tissue-cultured *Dendrobium officinale*; W-DHS = wild *D. huoshanense*; W-DM = wild *D. moniliforme*; W-DO = wild *D. officinale*.

Table 2 – Mean relative concentrations (expressed as % from total peak areas) and standard deviations of volatile compounds from different provenances of *Dendrobiums* analyzed by HS-GC/MS technique.

No	Chemical	RM	RI	TC-DHS	W-DHS	TC-DO	W-DO	TC-DM	W-DM
1	Cyclohexene[a,b,c]	874	704	—	—	—	—	6.15 ± 1.25	—
2	Methyl isobutyl ketone[a,b]	832	721	—	—	0.24 ± 0.07	—	—	6.38 ± 0.87
3	3-hydroxy-2-butanone[a,b,c]	842	733	3.53 ± 0.21	—	—	—	—	2.17 ± 0.11
4	N,N-dimethyl-propanamide[a]	803	741	—	1.09 ± 0.08	12.64 ± 1.19	0.14 ± 0.08	—	—
5	Not identified	—	752	—	—	—	1.98 ± 0.17	—	—
6	3-methyl-1-butanol[a,b,c]	819	762	—	—	—	—	—	0.86 ± 0.08
7	1-pentanol[a,b,c]	915	775	—	5.17 ± 0.55	—	—	—	—
8	Not identified	—	779	—	—	4.41 ± 0.23	—	4.64 ± 0.77	2.04 ± 0.24
9	1-octene[a,b]	821	785	—	—	0.61 ± 0.05	—	1.33 ± 0.27	0.69 ± 0.07
10	Hexanal[a,b,c]	914	790	4.84 ± 0.15	2.01 ± 0.07	10.39 ± 0.62	8.59 ± 0.33	12.95 ± 2.56	5.14 ± 0.88
11	(S)-2-hydroxypropanoic acid[a]	807	801	—	—	—	1.21 ± 0.25	—	—
12	Cyclopentanol[a,b,c]	896	803	—	—	1.4 ± 0.09	—	—	—
13	2,3-butanediol[a,b,c]	878	806	—	—	—	0.41 ± 0.11	—	—
14	4-methyloctanoic acid[a]	952	820	—	0.42 ± 0.04	0.57 ± 0.06	0.79 ± 0.14	0.16 ± 0.07	0.32 ± 0.05
15	Furfural[a,b]	878	833	2.48 ± 0.41	10.16 ± 1.18	11.75 ± 0.61	20.84 ± 1.75	4.68 ± 0.85	4.95 ± 0.66
16	3-methyl-1-pentanol[a,b]	887	846	—	—	1.66 ± 0.11	—	—	—
17	Not identified	—	857	—	—	0.13 ± 0.02	1.94 ± 0.22	—	—
18	2-heptanone[a,b]	875	873	—	0.15 ± 0.03	0.46 ± 0.05	0.24 ± 0.06	0.26 ± 0.09	0.13 ± 0.02
19	2-ethyl-heptanoic acid[a,b]	842	877	—	0.26 ± 0.04	0.9 ± 0.04	0.71 ± 0.08	1.12 ± 0.07	1.71 ± 0.15
20	2-hexen-1-ol, (E)-[a,b]	921	881	—	—	0.53 ± 0.05	—	—	—
21	Not identified	—	895	—	—	—	—	1.21 ± 0.18	1.06 ± 0.09
22	Not identified	—	899	—	—	0.49 ± 0.08	0.63 ± 0.07	—	—
23	11-hexadecen-1-ol, (E)-[a]	809	961	0.92 ± 0.06	0.56 ± 0.04	—	—	1.08 ± 0.11	—
24	Benzaldehyde[a,b]	933	982	—	—	1.03 ± 0.11	1.28 ± 0.22		
25	4,4-dimethyl-2-cyclohexen-1-one[a]	815	992	—	—	2.89 ± 0.78	—	—	—
26	Benzeneacetaldehyde[a,b]	903	1003	8.05 ± 1.33	9.44 ± 0.87	6.64 ± 1.12	6.5 ± 0.75	11.01 ± 1.03	9.78 ± 0.94
27	Cis-9-tetradecen-1-ol[a]	824	1014	0.34 ± 0.06	—	—	—	0.59 ± 0.08	—
28	Eucalyptol[a,b]	847	1032	6.14 ± 1.47	2.39 ± 0.54	10.31 ± 1.96	10.79 ± 1.32	7.98 ± 0.85	3.02 ±
29	Not identified	—	—	—	—	0.88 ± 0.21	—	—	—
30	1-octanol[a,b]	839	1051	—	1 ± 0.11	3.8 ± 0.96	2.29 ± 0.77	—	0.18 ± 0.03
31	Cyclopentadecanol[a]	804	1061	2.28 ± 0.22	0.7 ± 0.05	1.3 ± 0.23	1.18 ± 0.21	1.6 ± 0.08	3.44 ± 0.39
32	Acetophenone[a,b]	856	1069	0.48 ± 0.08	0.16 ± 0.06	0.38 ± 0.11	0.46 ± 0.08	0.77 ± 0.09	0.49 ± 0.07
33	1-octanol[a,b,c]	897	1083	—	—	0.69 ± 0.12	0.64 ± 0.07	0.46 ± 0.06	—
34	Terpinolene[a,b,c]	868	1097	11.78 ± 2.13	2.65 ± 0.39	1.46 ± 0.32	5.62 ± 0.99	28.18 ±	11.22 ± 1.22
35	Nonanal[a,b]	884	1108	0.71 ± 0.09	—	0.22 ± 0.08	0.23 ± 0.07	0.61 ± 0.04	—
36	Linalool[a,b]	981	1114	0.52 ± 0.07	3.22 ± 0.58	2.07 ± 0.55	9.46 ± 0.82	—	—
37	Nonanal[a,b,c]	947	1128	—	—	—	—	0.24 ± 0.03	2.37 ± 0.15
38	1-acetyl-2-methyl-1-cyclopentene[a]	936	1135	—	0.3 ± 0.05	0.33 ± 0.04	0.26 ± 0.07	0.48 ± 0.08	0.41 ± 0.07
39	Undecanal[a]	892	1151	0.88 ± 0.06	0.6 ± 0.04	—	—	—	—
40	3-ethyl-phenol[a,b]	845	1165	—	0.69 ± 0.09	0.73 ± 0.11	5.33 ± 0.88	—	0.54 ± 0.09
41	Not identified	—	1171	—	0.75 ± 0.08	0.99 ± 021	0.89 ± 0.09	—	—
42	1-dodecene[a,b]	848	1175	18.56 ± 2.35	2.08 ± 0.79	0.88 ± 0.16	2.51 ± 0.44	0.48 ± 0.08	1.68 ± 0.11
43	Octanoic acid[a,b]	854	1185	14.07 ± 1.15	2.5 ± 0.57	3.07 ± 0.22	2.81 ± 0.45	4.22 ± 0.77	9.98 ± 0.65
44	A,4-dimethy l-3-cyclohexene-1-acetaldehyde[a]	817	1184	—	—	—	0.34 ± 0.05	—	—
45	Glycolophenone[a]	832	1193	0.69 ± 0.05	0.12 ± 0.05	0.47 ± 0.13	0.44 ± 0.08	0.74 ± 0.08	3.52 ± 0.54
46	Octanoic acid ethyl ester[a,b]	899	1203	7.85 ± 1.87	1.99 ± 0.15	2.47 ± 0.22	2.01 ± 023	2.61 ± 0.21	14.24 ± 2.13
47	4-terpinenol[a,b]	821	1220	—	1.08 ± 0.13	0.81 ± 0.09	1.02 ± 0.08	—	0.46 ± 0.05
48	Not identified	—	1234	0.75 ± 0.11	1.41 ± 0.21	1.27 ± 0.22	1.49 ± 0.17	0.44 ± 0.07	1.07 ± 0.05
49	Isolongifolene epoxide[a]	809	1253	—	—	—	—	0.12 ± 0.04	—
50	2,3,5-trimethyl-phenol[a,b,c]	884	1266	2.13 ± 0.09	—	—	—	—	—
51	1-decanol[a,b]	875	1285	0.69 ± 0.07	41.4 ± 3.87	—	—	—	—
52	9-tetradecen-1-ol, (E)-[a]	855	1295	—	—	0.65 ± 0.07	—	—	1.98 ± 0.21
53	3,4-dihydro-5,7-dimethyl-1(2H)- Naphthalenone[a]	801	1311	—	—	—	0.76 ± 0.08	—	—
54	2-naphthaleneethanol[a]	832	1329	0.5 ± 0.1	0.26 ± 0.04	0.17 ± 0.03	—	0.21 ± 0.04	1.15 ± 0.14
55	1-hexadecen-1-ol, (Z)-[a]	896	1347	—	0.81 ± 0.08	—	—	—	—
56	1-undecanol[a,b]	854	1368	—	0.68 ± 0.11	—	—	—	—
57	Not identified	—	1396	—	0.1 ± 0.02	0.15 ± 0.04	0.19 ± 0.05	0.13 ± 0.03	0.34 ± 0.07
58	Dodecanal[a,b,c]	875	1426	0.25 ± 0.06	0.15 ± 0.03	0.18 ± 0.05	—	0.09 ± 0.02	0.73 ± 0.09
59	Not identified	—	1488	—	—	0.58 ± 0.07	0.21 ± 0.04	—	—

(*continued on next page*)

Table 2 – (continued)

No	Chemical	RM	RI	TC-DHS	W-DHS	TC-DO	W-DO	TC-DM	W-DM
60	2-naphthalenol[a,b]	855	1524	—	0.14 ± 0.03	0.11 ± 0.05	—	—	0.24 ± 0.07
61	N-tridecanol[a,b,c]	891	1591	4.42 ± 0.24	—	—	—	—	—
62	Not identified	—	1675	—	—	—	—	—	0.63 ± 0.09
63	Pentadecanoic acid[a,b]	826	1889	2.39 ± 0.31	—	—	—	—	—

— = not detected in our experimental conditions; HS-GC/MS = headspace gas chromatography–mass spectrometry; RI = retention indices; RM = relative match; TC-DHS = tissue-cultured *Dendrobium huoshanense*; TC-DM = tissue-cultured *Dendrobium moniliforme*; TC-DO = tissue-cultured *Dendrobium officinale*; W-DHS = wild *D. huoshanense*; W-DM = wild *D. moniliforme*; W-DO = wild *D. officinale*.

[a] Tentatively identified based on the National Institute of Technology and Standards library.

[b] Identified based on the RI in literature.

[c] Identified based on the authentic chemicals.

The GC–MS analysis reflected the low boiling point chemical constitutes of *Dendrobium* (the volatile metabolites), which are usually influenced by not only the genetic materials but also the growing conditions [16–21]. Our experiments revealed that the accumulation of the volatile metabolites in *Dendrobiums* might be affected by their provenances to a much greater extent than we had expected. For medicinal plants, the volatile compounds might originate in three possible ways: the metabolism of the plants themselves, the metabolism of the endophytic fungi in the medicine plants, or the defensive substance of the endophytes and/or the host plants. For the three investigated tissue-cultured *Dendrobiums*, removing the endophytic fungi in the sterile stage of test-tube seeding might cause the absence of the endophyte-originated metabolites in the plants, although more experimental data are still needed. Keeping the chemicals similar between the tissue-cultured medicinal plants and their wild correspondences is a challenge and bottleneck in the conservation and utilization of the endangered *Dendrobium* species, especially *D. huoshanense*, using tissue culture technology.

3.3. Similarity analysis of GC–MS fingerprint of different provenances of dendrobiums

The similarity evaluation based on chromatographic fingerprint analysis of different samples is a common technique [22]. To evaluate further the similarity of different provenances of *Dendrobiums*, the correlation coefficient calculated by included cosine angle was used as a similarity measure in the present study to appraise the similarity of the six *Dendrobium* samples. As shown in Table 3, different provenances of *Dendrobium* samples exhibited rather low similarity with the correlation coefficients 0.7–0.8, indicating that the three investigated *Dendrobiums* had some specific volatile compounds between different provenances, although they obtained the similar genetic information and cultivated on the same conditions. Furthermore, the correlation coefficients of different species were obviously lower than those between different origins consisted with the same genetic information between different origins while much more differences among different species. Li et al [23] had reported that the genetic distance between *D. moniliforme* and *D. huoshanense* was smaller than that between *D. huoshanense* and *D. tosaense*, and *D. huoshanense* should be the synonym of *D. moniliforme*. Our study showed that the coefficients of TC-DHS and W-DM were much higher than those of DHS and DO, which might provide further evidence for Li et al's suggestion [23].

Table 3 – The correlation coefficients based on chromatographic fingerprint analysis of the six *Dendrobium* samples.

	TC-DHS	W-DHS	TC-DO	W-DO	TC-DM	W-DM
TC-DHS	1.000	0.726	0.394	0.438	0.596	0.720
W-DHS		1.000	0.247	0.295	0.485	0.558
TC-DO			1.000	0.765	0.471	0.473
W-DO				1.000	0.533	0.501
TC-DM					1.000	0.790
W-DM						1.000

TC-DHS = tissue-cultured *Dendrobium huoshanense*; TC-DM = tissue-cultured *Dendrobium moniliforme*; TC-DO = tissue-cultured *Dendrobium officinale*; W-DHS = wild *D. huoshanense*; W-DM = wild *D. moniliforme*; W-DO = wild *D. officinale*.

3.4. PCA

Similarity analysis embodies the characteristics of GC–MS fingerprint with *integrity* and *fuzzy* [24]. However, this analytical method cannot clearly show the relationships among nonadjacent objects [24,25]. In order to discriminate the investigated *Dendrobiums* more clearly, PCA of the data was performed by taking into consideration the volatile profiles of both wild and tissue-cultured *Dendrobium* samples.

The PCA (Figures 2 and 3) results of the first three principal components explained 85.9% of the variance of the data, showing a good discrimination between the samples. The score plot and the loading plot gave us valuable information regarding the correlation between *Dendrobiums* and volatile compositions to reveal the correlation between variables (volatile compounds and *Dendrobium* species and provenance). Thus, based on their volatile profiles, TC-DHS, TC-DO, and TC-DM could be discriminated easily from their wild correspondences W-DHS, W-DO and W-DM (Figure 2). Also, the volatile profile of TC-DHS was similar to the volatile profile of *D. moniliforme* samples. The loading plot of the three first components (Figure 3) showed a strong relationship between five major compounds 44, 29, 15, 23, and 37 (nonanal) in *Dendrobiums*.

4. Conclusions

The use of GC–MS combined with chemometrics to analyze the volatile compounds of *Dendrobiums* can effectively classify

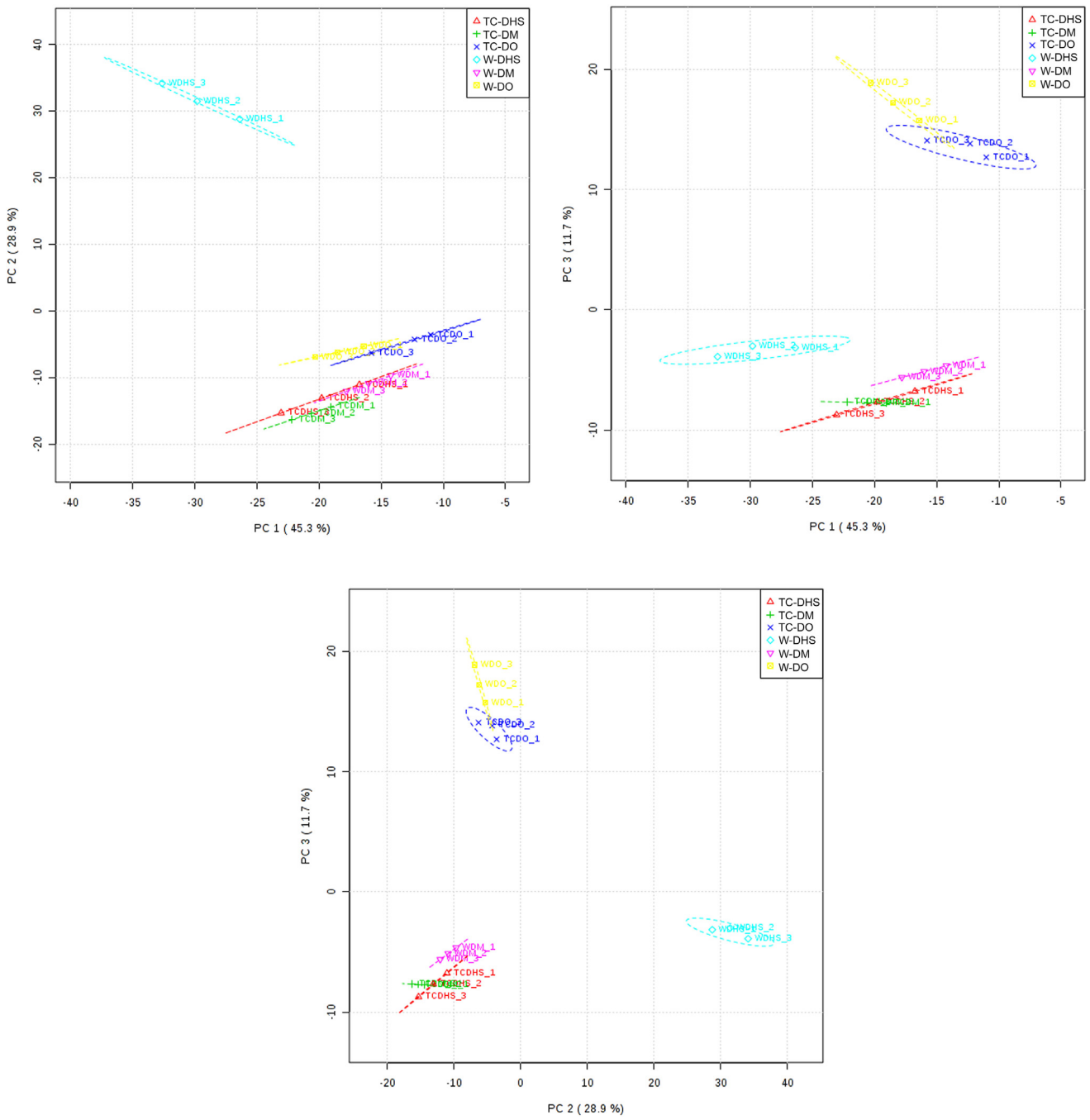

Figure 2 – Two-dimensional score-plots of principal components. TC-DHS = tissue-cultured *Dendrobium huoshanense*; TC-DM = tissue-cultured *Dendrobium moniliforme*; TC-DO = tissue-cultured *Dendrobium officinale*; W-DHS = wild *D. huoshanense*; W-DM = wild *D. moniliforme*; W-DO = wild *D. officinale*.

and identify the three investigated *Dendrobium* species of different provenances and suggest reasons for their varying chemical compositions. The results obtained in this study can provide a comprehensive evaluation for the quality of *Dendrobiums* of different provenances and an optimization evaluation method for medicinal herb quality control.

By using PCA as well as similarity evaluation based on the GC–MS data, it was revealed that the qualitative compositions and the contents of the volatile compounds in *Dendrobiums* were generally characteristic and specific between tissue-cultured and wild stocks; this suggests that the chemical constituents in tissue-cultured *Dendrobiums* are quite different from those in their corresponding wild stocks although they have similar genetic information and homoplastic morphologies and were cultivated in the same environmental conditions. In addition, further investigations, such as whether the variation in chemicals between tissue-cultured and wild *Dendrobiums* would increase or decrease their

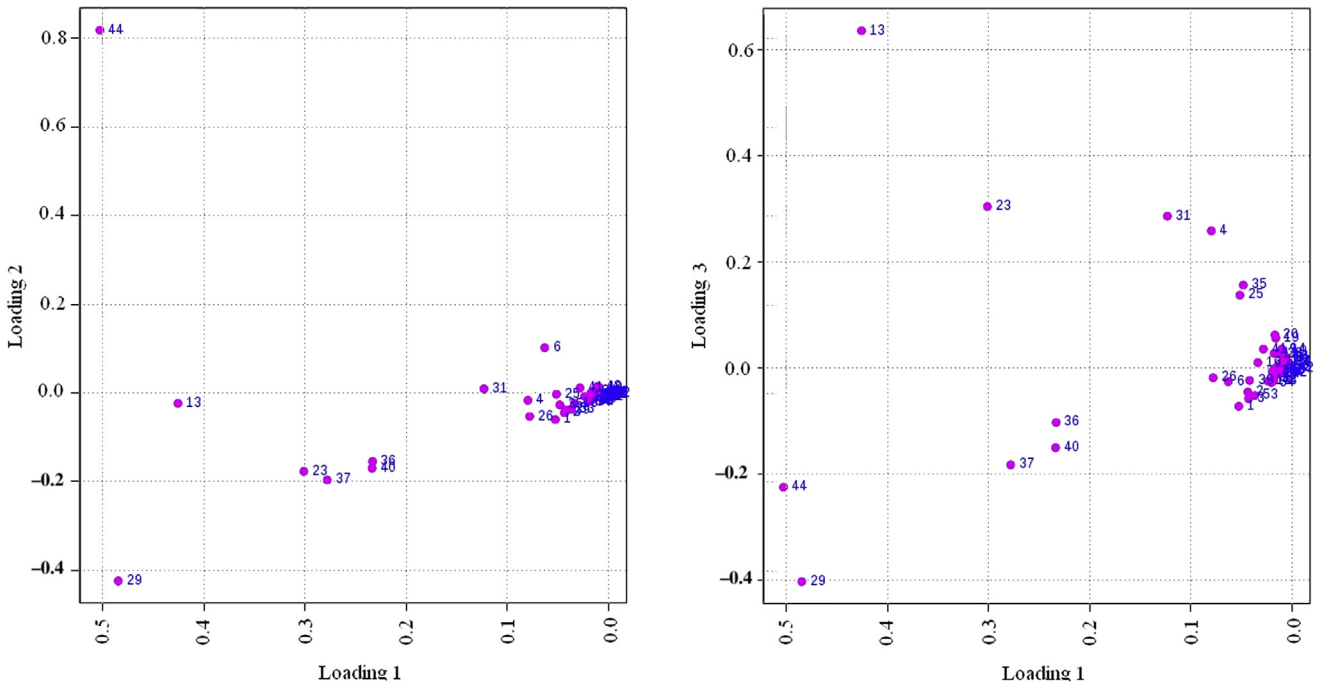

Figure 3 – The correlation loadings biplots for tissue-cultured and wild *Dendrobium* varieties. The numbering refers to Table 2.

pharmacodynamic action, are critically needed for the conservation and utilization of rare and endangered *Dendrobiums*.

Conflicts of interest

All authors have no conflicts of interest to declare.

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